Prostaglandin Concentrations in Uterine Fluid of Cows with Pyometra

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ABSTRACT

Uterine fluid was obtained from eight clinical cases of pyometra with retained corpus luteum and nine additional samples of fluid were collected from animals slaughtered at an abattoir. These samples, along with uterine flushes from normal cows in their luteal phase were analyzed for prostaglandin of the F (PGF) and E (PGE) groups. Blood samples were also obtained from the clinical cases for analysis of 13,14-dihydro-15-keto PGF (PGFM) the major metabolite of PGF. Pyometrial exudate from clinical cases of abattoir samples had high concentrations of PGF (17.9 ng/mL) and PGE (33.2 ng/mL) and the total amount of PGF and PGE in the uterus was calculated to be several hundred times as great as in normal cows. Furthermore, clinical cases had elevated PGFM in their blood compared to that of controls, which suggests that at least some of the PGF was being absorbed from the uterus. These results are discussed in light of our current understanding of the maternal recognition of pregnancy in cattle.

Key words: Pyometra, prostaglandin, cows.

RÉSUMÉ

Cette expérience consistait à récolter de l'exsudat utérin, chez huit vaches atteintes de pyomètre et de rétention du corps jaune, ainsi que des sécrétions utérines, chez neuf vaches envoyées à l'abattoir, au cours du dioestrus. On rechercha, dans ces échantillons et dans la solution saline avec laquelle on avait lavé la muqueuse utérine de vaches abattues au cours du dioestrus, les prostaglandines des groupes F et E. On préleva aussi du sang, chez les vaches atteintes de pyomètre, afin d'y rechercher le principal métabolite de la prostaglandine F, la 13, 14-dihydro-15-céto-prostaglandine. L'exsudat utérin des vaches atteintes de pyomètre et les sécrétions utérines des vaches envoyées à l'abattoir contenaient autant que 17,9 ng/mL de prostaglandine F et 33,2 ng/mL de prostaglandine E; la quantité totale de ces deux variétés utérines de prostaglandines s'avéra plusieurs centaines de fois supérieure à celle des vaches normales. De plus, les vaches atteintes de pyomètre recelaient un taux sanguin plus élevé du métabolite précité que les témoins, indice qu'au moins une certaine partie de la prostaglandine F était absorbée à partir de l'utérus. Les auteurs commentent leurs résultats à la lumière de notre compréhension actuelle de la reconnaissance maternelle de la gestion, chez la vache.

Mots clés: pyomètre, prostaglandine, vaches.

INTRODUCTION

Pyometra is a condition which occurs frequently in cows during the postpartum period. It is characterized by uterine infection, accumulation of a purulent exudate and persistence of the corpus luteum (1). The causes of uterine infection and the organisms involved can be numerous; however, the reasons for retention of the corpus luteum (CL) are not understood.

Numerous studies have shown that prostaglandin $F_{2\alpha}$ (PGF) and its analogues are potent luteolytic agents (2,3) and the concept has evolved that CL maintenance in pregnancy is due, in part, to inhibition of uterine production of PGF (4). However, most workers have not detected differences in utero-ovarian vein PGF in pregnant compared to nonpregnant ewes (5,6,7,8) and current results suggest that sheep and cattle utilize similar mechanisms for maternal recognition of pregnancy (9,10). Furthermore, recent studies have shown that 15-16 day old sheep embryos produce, and apparently secrete several proteins, one of which can extend CL lifespan when infused into the uterus of nonpregnant animals (11). Also, prostaglandin E_1 and E_2 can extend CL lifespan when infused into the uterus of sheep (12) and cattle (13) and sheep embryos synthesize both PGE and PGF *in vitro* (14).

Thus, it seems likely that the embryo, during the critical period of maternal recognition of pregnancy (day 14 to approximately day 20 after mating), produces a protein(s) which could reduce uterine PGF production, or cause a shift to synthesis of the luteotropic prostaglandins (PGE). Increased PGE production could alter the balance to favour luteal maintenance. A previous study (15) demonstrated elevated concentrations of PGF and PGE in pyometrial fluid in three cases. It seemed worthwhile to confirm and extend that observation in a larger number of cows with pyometra.

In the present study, samples of uterine fluid from cows with pyometra were analyzed for concentrations of PGE, PGF and PGFM and compared to normal animals. Where possible, comparisons were made between blood and uterine concentrations and the information is interpreted in the light of our present understanding of maintenance of luteal function.

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MATERIALS AND METHODS

SAMPLES OF THE UTERINE FLUID

Eight clinical cases of pyometra with CL retention were sampled. These animals were at least three months postpartum and were diagnosed because the owner wished to breed them but was unable to detect them in estrus. A Foley catheter was inserted into the uterus and 10-30 mL of purulent material was aspirated. A jugular vein blood sample was also collected and the serum was frozen. Nine additional cases which met the following criteria were selected from an abattoir: nonpregnant, a substantial volume of uterine fluid, a mature nonregressed CL which seemed functional. In these cases, samples of uterine fluid were taken with a large diameter needle through the uterine wall. All samples were chilled after collection and centrifuged at 1800 x g for 10 min to yield a clear fluid which was stored at -20° C until analysis. In addition, abattoir material was used to select uteri from apparently normal cows with a mature nonregressed corpus luteum; these uteri were flushed with 20 mL saline and this flushing fluid was centrifuged and saved for analysis.

PROSTAGLANDIN ASSAYS

The assays for PGE and PGF have been described (14). Results are expressed as PGE and PGF because the antisera did not differentiate between PGE_1 and PGE_2 nor between $PGF_{2\alpha}$ and $PGF_{1\alpha}$. Briefly, samples were acidified with acetic acid and then extracted with eight volumes of petroleum ether to remove neutral lipids. The remaining aqueous phase was then extracted into five volumes of ethyl acetate and that solvent was dried down. The samples were redissolved in 0.2 mL benzene: ethyl acetate:methanol (B:EA:M, 60:40:10) and applied to a small florisil column to separate the PGE from the PGF group of prostaglandins. After appropriate washing to remove unwanted fractions, PGE was removed with 8 mL of B:EA:M (60:40:2.4) and PGF with 2 mL of B:EA:M (60:40:20). Recoveries (%) after extraction and chromatography were determined using radioactive PGE and PGF and averaged 68 ± 2 and 76 ± 2 for PGE and PGF respectively. Concentrations of PGE and PGF were measured in two separate radioimmunoassays, one assay used a PGE antibody and ^3H-PGE_2 as tracer; the other used a PGF antibody and ^3H-$PGF_{2\alpha}$ as tracer. Intraassay and interassay coefficients of variation were similar for PGE and PGF and were 9.9 and 13.7% respectively.

In addition jugular vein serum was assayed for 13,14-dihydro-15-keto $PGF_{2\alpha}$ (PGFM) the major metabolite of PGF which has a much longer half-life in blood than PGF and which is frequently used to reflect PGF production. This radioimmunoassay was done on unextracted serum as described by Manns *et al* (14).

STATISTICAL METHODS

Data are expressed as means ± standard error; differences were evaluated by unpaired "Student's" t tests.

RESULTS

Uterine fluid from cows with pyometra had extremely high concentrations of PGE and PGF (Table I). For comparison, uterine flushes from normal cows are compared to cows with pyometra (Table I). It is not possible to obtain significant quantities of uterine fluid from the normal diestrus uterus hence, concentrations in the fluid coating the endometrial cells would be much higher than those shown in Table I. However, the PGE/PGF ratio in normal cows compared to cows with pyometra was significantly different ($P < 0.05$). Although the total amount of pyometrial fluid was not measured, it was greater than 100 mL in each cow. Based on that minimum value, the total uterine content of PGE and PGF in cows with pyometra would be 1800 and 3300 ng. In normal cows the comparable values would have been approximately 4 and 10 ng respectively.

Table I lists values for PGFM in plasma of normal cows and in cows with pyometra, and in the uterine fluid of cows with pyometra. The PGFM was higher in cows with pyometra ($P < 0.01$) and there was a strong correlation ($r = 0.87$) between uterine PGF and plasma PGFM.

DISCUSSION

The uterine fluid in cows with pyometra contains high concentrations and large total amounts of PGF and PGE. The concentrations measured in the present study indicate that the total uterine contents of PGF could easily exceed 10 μg. It is known that a single intrauterine deposition of 500 μg PGF can induce luteolysis (2) and, if rates of PGF synthesis are high due to the inflammatory process (18) it is possible that amounts approaching a luteolytic dose could be synthesized. It is also possible that the PGF synthesized is confined to the uterus, however, the fact that cows with pyometra also had elevated plasma concentrations of the metabolite PGFM suggests that some absorption was occurring. Furthermore, Thatcher *et al* (19) have shown that in normal animals, PGE will enhance absorption of PGF from the uterus. Whether such

TABLE I. Mean Concentration of Prostaglandins in Blood and Uterine Fluids from Normal Cows and Cows with Pyometra. Variability Around the Mean is Shown by Standard Errors (± SEM) as Well as Ranges for Some Values

	Animals Studied			
		Pyometra		
	Normal	Clinical Cases	Abattoir	Total
Number of cows	8	8	9	17
Uterine PGE (ng/mL)	0.20 ± 0.03	19.2 ± 2.9	16.7 ± 3.3	17.9 ± 2.2
Range	0.12-0.27	5.8-31.3	5.6-34.9	5.6-34.9
Uterine PGF (ng/mL)	0.48 ± 0.03	34.3 ± 7.6	32.3 ± 6.7	33.2 ± 4.9
Range	0.38-0.75	11.0-75.8	6.9-65.7	6.9-75.8
Uterine PGE/PGF	0.42 ± 0.03[a]	0.64 ± 0.09[c]	0.61 ± 0.09[c]	0.63 ± 0.05[b]
Plasma PGFM (ng/mL)	0.31 ± 0.05	1.26 ± 0.14		

a vs b, $p < 0.05$, c — ratios for clinical cases and abattoir cows were similar

an effect would occur in cows with pyometra is not known.

The source of uterine fluid PGF and PGE could be from either the uterine wall or from leukocytes and/or bacterial organisms in the uterus. It seems likely (Nkuuhe, unpublished) that the primary source is macrophages, lymphocytes and neutrophils since *in vitro* cultures of such cells produce large amounts of PGF and PGE.

Considering the fact that there is such a large amount of PGF present, it seems paradoxical that the condition causes luteal maintenance. On balance, other studies (5,6,7,8,19,20) indicate that basal levels of uterine production of PGF are similar between pregnant and nonpregnant animals but that during the period of luteal regression nonpregnant animals have major spikes of secretion of PGF whereas pregnant animals do not. The possible luteotropic role of PGE which is produced in significant quantities by embryos (14) is not clear nor is the function of protein secreted by embryos (11). It is possible that the leukocyte and endometrial PGF can not be absorbed or, that the luteotrophic PGE compounds abrogate the effect of elevated PGF. If this is true it suggests a different cause for this condition than we have believed previously and implies that CL maintenance is induced by an active, positive signal as opposed to lack of a luteolytic signal. However since exogenous PGF will cause luteolysis in cows with pyometra (15), the balance between the luteotropic and luteolytic factors is critical and in that respect uterine infections may create conditions which are somewhat analogous to those during the period of maternal recognition of pregnancy when the developing embryo appears to be capable of altering prostaglandin production by the uterus.

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